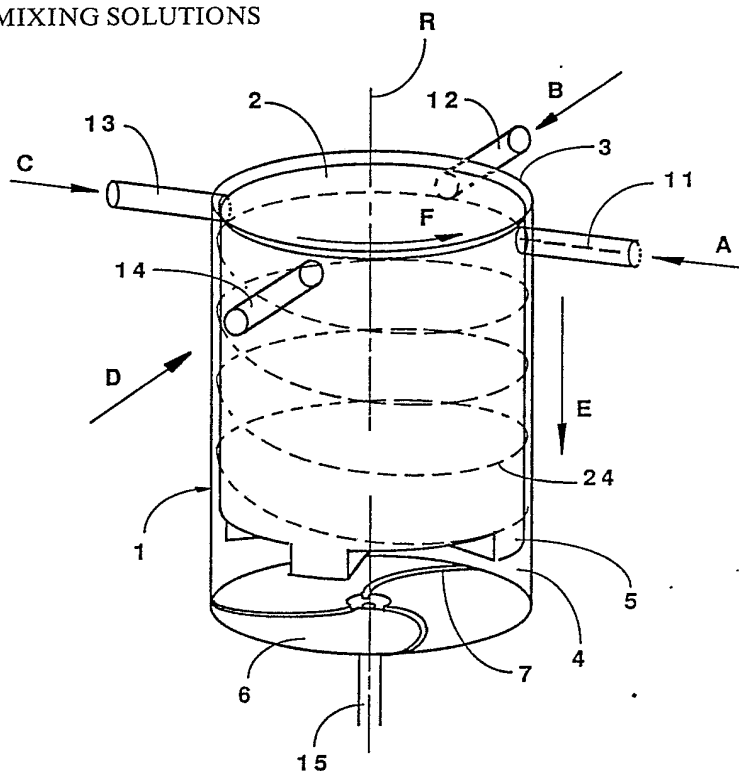




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(21) International Application Number: PCT/US89/00346 (22) International Filing Date: 27 January 1989 (27.01.89) (31) Priority Application Number: 321/88-4 (32) Priority Date: 29 January 1988 (29.01.88) (33) Priority Country: CH (71) Applicant: APPLIED BIOSYSTEMS, INC. [US/US]; 850 Lincoln Centre Drive, Foster City, CA 94404 (US). (72) Inventor: MICHEL, Bruno ; Obstgartenweg 13, CH-8136 Gattikon (CH). (74) Agent: SMITH, Joseph, H.; Wiseman, Jones & Smith, 12 S. First Street, Suite 911, San Jose, CA 95113 (US).	(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent). Published <i>With international search report.</i>	

(54) Title: DEVICE FOR MIXING SOLUTIONS**(57) Abstract**

A device for mixing liquids contains a mixing chamber (1) into which a mixing head propelled around a rotationally axis is designed. Between the wall of the mixing chamber and the mixing head (2) a cleft mixing area is created. Through rotation of the mixing head (2) a shear flux of the injected liquids (11, 12, 13, 14) is created which causes mixing. With this active mixing method minute liquid amounts with differing volumes and viscosities can be mixed with high rapidity. The device is especially suitable for the automated analysis of enzyme kinetics.

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DEVICE FOR MIXING SOLUTIONS

The present invention deals with a device for the mixing of solutions with a mixing chamber having minimally two input lines and an exit line.

Numerous chemical and biochemical analysis procedures are based on mixing exactly defined amounts of two or more solutions together and through observing the reaction conclusions about the characteristics of the liquid components drawn.

The characteristics of the to be analyzed solutions are dependent upon numerous parameters and, since problem-free analysis usually takes hundreds of independent measurements, it is clear why the manual mixing of liquids is very time consuming and tiresome. Moreover, the manual mixing procedure is a major problem area, on one hand due to quantitatively inaccurate mixing and on the other hand because of the long times required for the mixing and measuring procedures with unstable materials. Very fast reactions can not be analyzed at all because of the

deadtime, ie., time between mixing and start of the analysis, required is minimally 5 seconds. A reduction of the total analysis volume and the frequently very expensive liquids is not realizable by manual mixing.

Known devices for the mechanical mixing of liquids consists, for example, of two syringes each filled with a liquid whose contents are simultaneously injected into a mixing chamber, mixed based on the resulting turbulence, and subsequently transferred to a cuvette. By this measure the deadtime can be reduced to less than 5 milli seconds. To guarantee sufficient mixing the liquid must be injected into the mixing chamber under relatively high pressure. Due to the high back pressure in the mixing chamber the titration accuracy is reduced which is quite detrimental when different liquids and large volume differences must be mixed. Therefore it is not possible to mix volume differences of 1:50 with sufficient accuracy. Mixtures

of liquids with different viscosities very often don't reach the required homogeneity.

The task of the present invention, therefore, is to create a device for the fast mixing of small amounts of liquids with quite different viscosities. This task has been solved by the features of the first patent claim.

The essential advantages of this invention are that because of the active mixing principle no high injection pressures of liquids are required, which guarantees basically constant titration accuracy whereby very high mixing ratios (up to 1:2000) are achieved. Due to the proposed cleft shear flux liquids with quite different viscosities can be mixed without problem. The location of the mixing head in the mixing chamber, as proposed in the invention, ensures moreover a small dead volume which has a desirable effect on analysis cost of expensive substances.

In the following an invention is described in a preferred version which is especially suitable

for measuring the kinetic constants of enzymes. The following shows:

Fig. 1 Schematic presentation of a preferred version of the mixing chamber of the device according to the invention;

Fig. 2a and 2b the progress of the shear flux in first mixing area;

Fig. 3. Cross sectional view through the preferred version of the device according to the invention;

Fig. 4a and 4b axial and radial sectional views of a second cuvette;

Fig. 5. and arrangement/layout for the automatic determination of enzyme kinetic constants using the proposed invention; and

Fig. 6. The connection of the mixing device with matching controller.

Fig. 1 shows schematically the principal arrangements of this invention. Inside a closed mixing chamber (1) is a mixing head (2) built in such a way that it can be propelled around an rotational axis (R) and with the inner wall of the mixing chamber (1) a

rotationally symmetrical cleft mixing area (3) is created. The inside of the mixing chamber (1), as well as the mixing head (2), are, as shown in Fig. 1, constructed as coaxial vertical cylinders. Other forms such as cones, partial cones or any ∞ other rotationally symmetric configuration are also applicable. The mixing chamber (1) has at one end, for example, four feeding lines (11, 12, 13, 14) through which different liquids (by enzyme kinetic determinations these are enzyme, substrate, buffer and activator/inhibitor) in the direction of the arrows A, B, C, D continuously inject into the first mixing area (3). The feed lines (11, 12, 13, 14) enter preferably mixing chamber (1) all at the same height and evenly spaced around the circumference. The injection pressure creates an axial flux (arrow E) which is based on the rotation of the mixing head (2) a tangential flux is overlaid. The overall result is a shear flux in the cleft mixing area (3), in which, for example, one injected volume element through line 11 mainly on spiral movement (24) around the mixing head (2) proceeds and

is thereby mixed with other liquids. To assure accurate mixing the speed of mixing head (2) has to be much faster than the axial flowrate of the liquid (up to 20 times faster).

Fig. 2a shows an enlarged axial section through the entrance part of the mixing area (3). A predetermined volume element (21) through line 11 injected liquid shows approximately an axial flow cross section (22). Fig 2b shows a respective radial cross section through the entrance part of the mixing area (3) to illustrate a momentary tangential cross section (23) of the volume element (21).

The described hollow cylindrical shear flux in the first mixing area (3) slows the flux minimally, therefor the liquids can be injected with low pressure unlike the known passive mixing devices. By this means the injected liquids can be precisely controlled in volume proportions up to 1:2000 with enough accuracy. Because the mixing procedure in the entire

mixing area occurs evenly, mixtures of liquids of different viscosity and consistency can be produced without problems. The arrangement of the mixing head (2) inside mixing head (1) helps to keep the dead volume of the entire device very small.

The axial expansion of mixing head (2) is preferably smaller than mixing chamber (1) so that a second mixing area (4) is created in which there already exists a turbulent flux caused by the rotation of mixing heads (2), in turn, causes additional mixing of the different liquids. To intensify this turbulence the bottom of mixing head (2) has special projections, the bottom (6) of mixing chamber (1) has furrows. In the middle of bottom (6) is an exit hole (15) through which the liquid exits the mixing chamber (1). It is understood that the mixing chamber can be equipped with multiple exit holes.

Fig. 3 shows the arrangement of the previously described mixing chamber in the system (10). An outer cover (41)

surrounds an inner case (42) of which the latter has a cylindrical hole which forms mixing chamber 1. Separated from mixing chamber (1) the inner case (42) contains a motor (32) and a coupled drive magnet (31). The mixing head (2) is, for example, a magnet covered with an inert material which is driven on the same rotational axis (R) by another magnet (31). Through the pull of the drive magnet (31) the magnetic mixing head (2) is pulled to the top of the mixing chamber (1) thereby forming a thin liquid film between the mixing head and the cover which serves as a lubricant. Between the outer cover (41) and the insulated inner chamber (42) is a thermostated chamber (43) and the liquids for mixing are transported through a connecting piece (44) into the thermostated chamber (43) which is filled with thermostated water to mixing chamber (1) (illustration only shows a single feed line 11). The thermostated water acts simultaneously as cooling for the heat produced by motor 32. The mixture exits through exit 15 and proceeds via a connecting piece 52 to a

flow through cuvette 51. Different physical and chemical parameters of the mixture can be measured in cuvette (51) with usual analytical instruments (ie., by optional and electrical procedures). The mixture flows through the cuvette (51) and goes via another connector (53) and a line (54) through the thermostated chamber (43) to a second cuvette (62) in which an addition measurement can be made with an additional measuring element (61). The mixture exits through a tube (16) and the connector 44. With the help of connectors (52 and 53) many different cuvettes (51) can be inserted into the outer shells (41).

The construction of the second cuvette (62) is illustrated in Fig 4a and 4b. Fig. 4a shows an axial cross section, Fig 4a a radial cross section through the outer cover (41) and the connected cuvette (62). A hole (64) is provided for an exchangeable measuring probe (61) (compare with Fig. 3). By putting the probe (61) in place the hole (64) will be closed so that the mixture passing through feeding line (54) and a connector (63) to cuvette (61) exits the opening (64) through a lateral opening (65).

The volume of the second mixing area (4) corresponds preferably with the one of cuvette (51). This creates a buffer zone in which a homogeneous mixture is produced even by non-continuous additions of small amounts of liquids. To adapt the volume of the second mixing area (4), and to provide variation for the cleft size of the first mixing area (3), an array of mixing heads are planned that can be simply exchanged by opening the mixing chamber (1) (ie., removal of the base (6), as shown in Fig. 3, is part of the screwed together outer case 41). To guarantee a constant cleft size during operation in the first mixing area (3) it is recommended to arrange the proposed device in such a way so that axial (R) is vertical manner. It does not matter which part of the device is on top. The chosen illustration in the figures is random because the flux of the liquids to the device is independent of gravity but is a matter of the injection pressure.

The proposed mixing device is especially useful for the automatic analysis

of enzyme kinetic constants where generally hundreds of single measurements are required. Fig. 5 shows a possible arrangement in which the proposed mixing device (10), including a cuvette (51), are put into a conventional analytical instrument (101). The proposed device can be designed in such a way that the use of conventional instruments is possible. The liquids of mixing come from a control unit (102), through the different feed lines inside of a surrounding tube (71), to connector piece (44) of the device (10). The control unit (102) has a sample chamber (105) from which the liquids are loaded by sucking syringes. The barrels of the syringes are equipped with, for example, stepper motors so that for each measurement the required liquid amount is exactly delivered through tube (71) into the mixing chamber (1) of the device (10). Sample chamber (105) can be additionally equipped with an autosampler so that one of the components can be automatically changed with each series of measurements. The analysis is controlled and results analyzed with a computer (104) equipped with the respective peripheral

instruments. An instrument (103) provides the previously mentioned thermostated water.

Fig. 6 shows the backside of the control unit (102) with a flexible tube (71) to mixing device (10). Through this tube run the four feeding lines (11, 12, 13, 14), the return (16) for the analyzed mixture, an electrical connecting piece (108) for temperature sensor (not shown), power line (109) for the drive motor (32), as well as a feeder line (17) for delivering thermostated water to the thermostated chamber (43). The return flow of the thermostated water in line (71) preferably occurs in an open manner such that the liquids in the feeding lines are already surrounded by thermostated water. This provides a very accurate temperature control of the components.

Patent Claims

1. Device for the blending (mixing) of solutions with a mixing chamber (1) having at least two solution inputs (11, 12, 13, 14) and one exit port (15), hereby shown that the interior of the mixing chamber is a rotating axel (R) with a mixing head, where the mixing chamber and head are designed such that they form at least a rotationally symmetric mixing area in which the rotating mixing head produces a shear mixing.

2. Device after claim 1 characterized by the mixer containing a second mixing area which produces a turbulent flow.

3. Device after above claims characterized by the interior of the mixing chamber and head being designed as vertical co-axial cylinders whereby

the mixing head is located within the mixing chamber, so the aforementioned first mixing area (3) is basically the form of a vertical cylinder formed between the surface of the mixing chamber and the mixing head.

4. Device based on claims 2 and 3 characterized by the cylinder height of the mixing head (2) being smaller than the interior of the mixing chamber with the mixing head attached at one end of the chamber so that the other end forms the aforementioned second mixing area (4) between the bottom of the mixing body, as well as the bottom and surface of the mixing chamber.

5. Device based on claim 4 characterized by minimally two supply lines leading (11, 12, 13, 14) preferably on the same level and evenly spaced around the circumference to the first mixing area (3) and that at least one fluid exit hole (15) in the second mixing area (4) is preferably located in the middle of the bottom surface of the mixing chamber.

6. Device based on claims 2 through 5 characterized in that the second mixing area (4) with the

mixing head (2) works together with the surfaces (5, 7) such that through rotation of the mixing head in the second mixing area a turbulent flow is created.

7. Device based on the above claims characterized by the mixing head (2) being made out of a magnet and that the drive magnet as well as drive motor is outside of the mixing chamber (1) on the same axis of rotation (R).

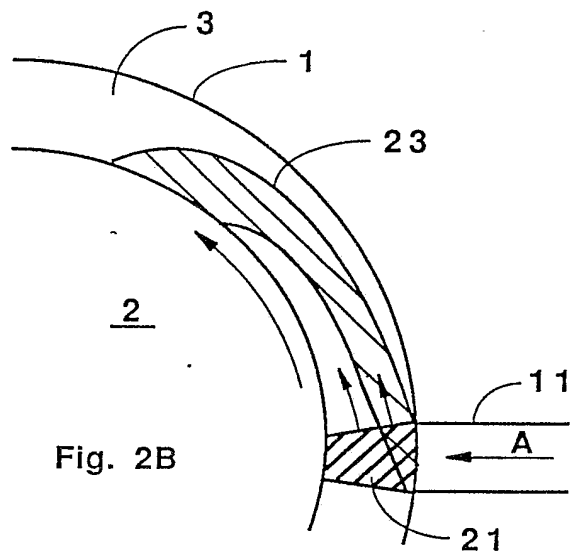
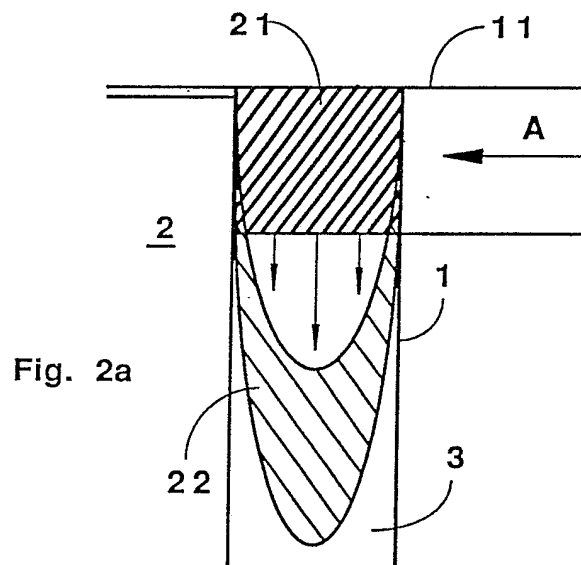
8. Device based on the above claims characterized by an interior chamber (42) containing mainly the mixing chamber (1) and drive (31, 32), as well as an exterior chamber (41), whereby between the two chambers a thermostated chamber (43) for controlling the temperature in the mixing chamber is connected to a water bath (17, 71, 102, 103).

9. Device based on the above claims characterized by having the supply lines (11, 12, 13, 14) from the control unit (102) for the controlled addition of liquids and

a piece (52) is foreseen to be the connection for minimally one liquid exit (15) to at least one cuvette (51) for analysis of the liquid mixtures.

10. Use of the device based on the previous claims for determining kinetic constants of enzymes where the cuvette (51) is placed in an analytical instrument/detector (101) and a control unit (102) measures determined amounts of enzymes, substrates, buffers and activator or inhibitor through the supply lines (11, 12, 13, 14) into the mixing chamber (1), mixed, and the mixture exists through the exit hole (15) of the mixing chamber (1) as well as the connecting lines (52, 53) through the cuvette (51).

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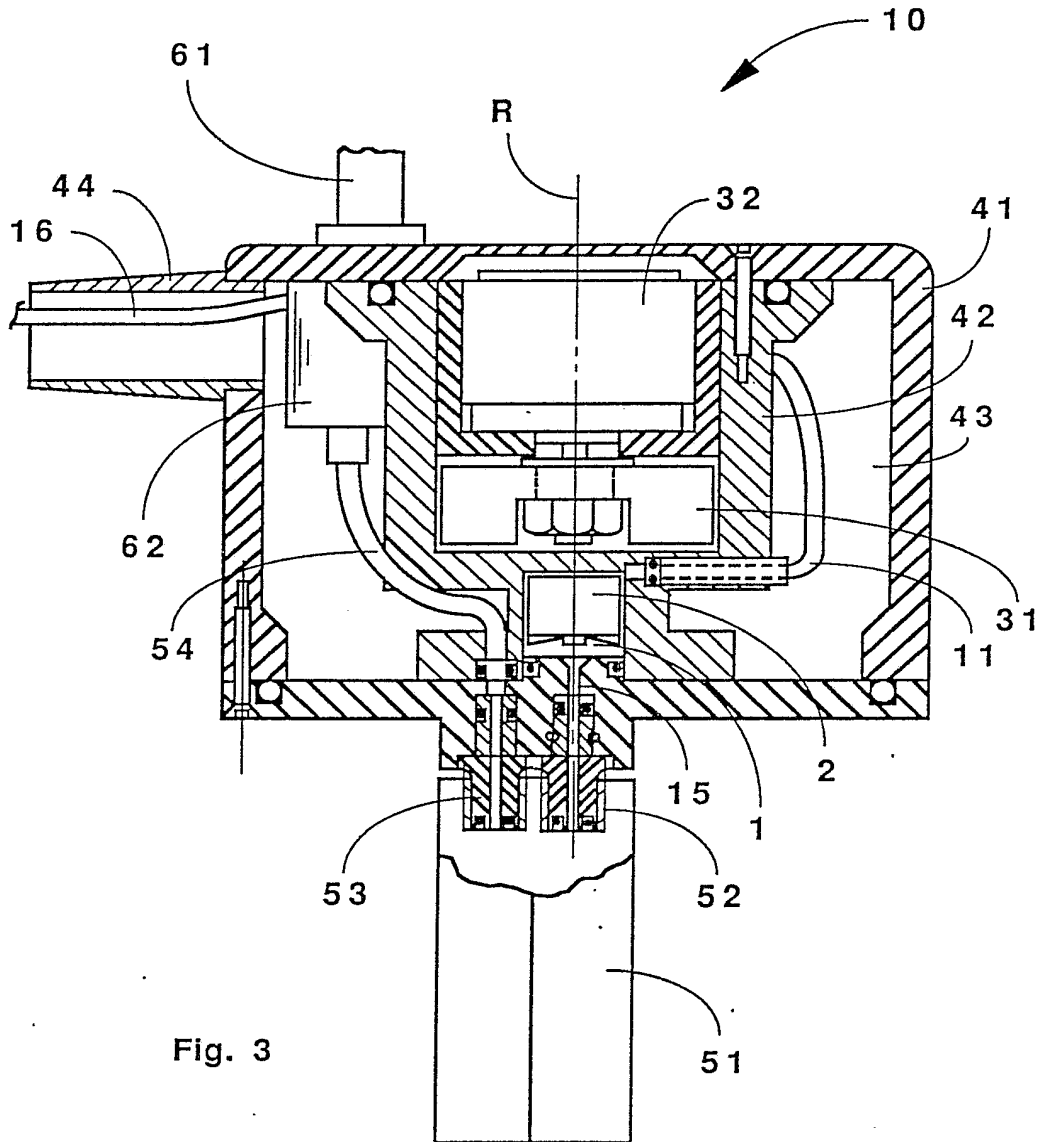


Fig. 3

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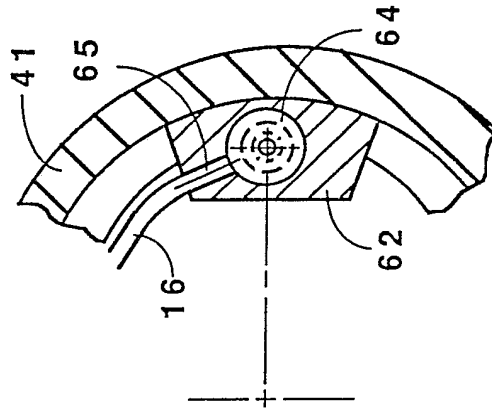


Fig. 4b

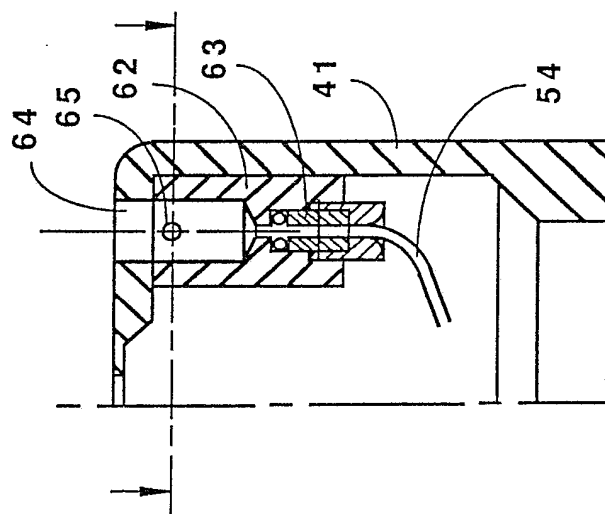
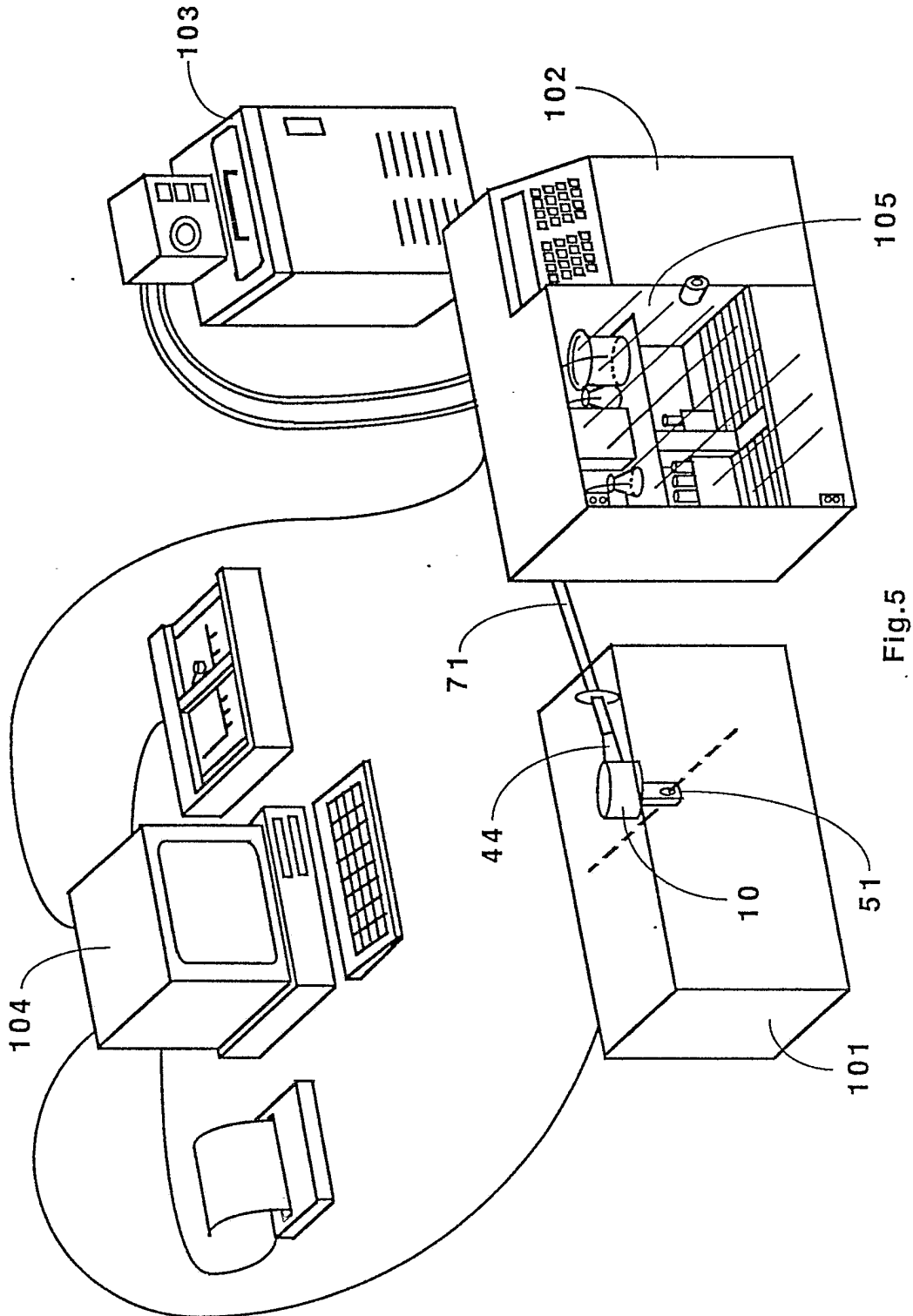
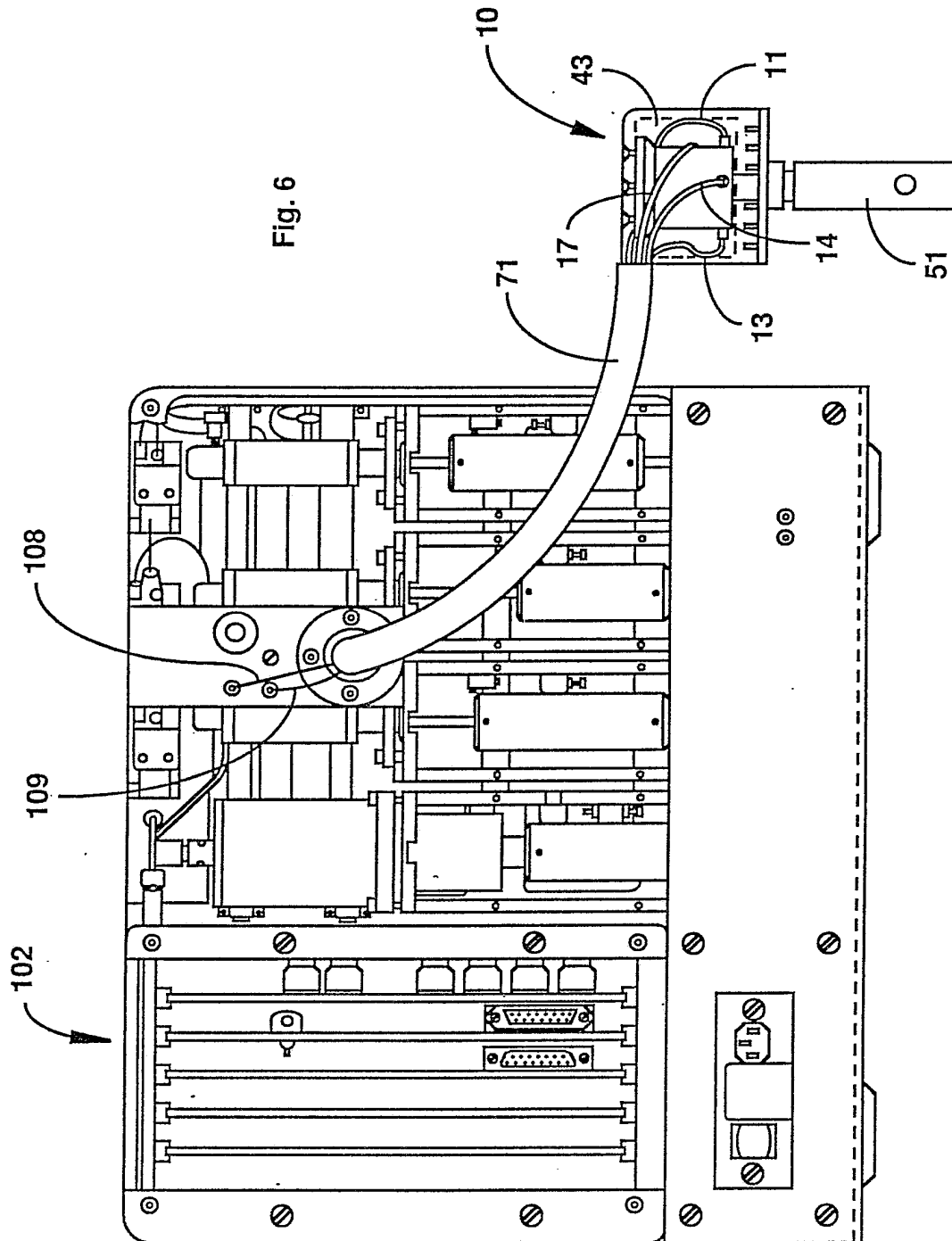


Fig. 4A



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Fig. 6



INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/00346

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC IPC(4): B01F 5/04; B01F 7/16; B01F 15/02; B01F 15/04; B01F 15/06 U.S. CL. 366/142, 149, 160, 165, 177, 273, 279																																
II. FIELDS SEARCHED <div style="text-align: center; border: 1px solid black; padding: 2px; margin: 5px 0;">Minimum Documentation Searched ⁷</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 20%; border: 1px solid black; padding: 2px;">Classification System</th> <th style="border: 1px solid black; padding: 2px;">Classification Symbols</th> </tr> <tr> <td style="border: 1px solid black; padding: 5px; vertical-align: top;">U.S.</td> <td style="border: 1px solid black; padding: 5px;"> 366/132, 134, 140, 142, 143, 144, 145, 149, 150, 152, 366/160, 162, 165, 167, 168, 173, 177, 178, 182, 184, 194 366/261, 273, 274, 286, 290, 279, 315-317, 349 </td> </tr> </table> <div style="text-align: center; border: 1px solid black; padding: 2px; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸</div>			Classification System	Classification Symbols	U.S.	366/132, 134, 140, 142, 143, 144, 145, 149, 150, 152, 366/160, 162, 165, 167, 168, 173, 177, 178, 182, 184, 194 366/261, 273, 274, 286, 290, 279, 315-317, 349																										
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III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹ <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%; border: 1px solid black; padding: 2px;">Category [*]</th> <th style="border: 1px solid black; padding: 2px;">Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²</th> <th style="border: 1px solid black; padding: 2px;">Relevant to Claim No. ¹³</th> </tr> <tr> <td style="border: 1px solid black; text-align: center; padding: 5px;">X</td> <td style="border: 1px solid black; padding: 5px;">US, A, 4482254 (Kessler et al) 13 Nov. 1984 See entire document</td> <td style="border: 1px solid black; text-align: center; padding: 5px;">1, 2</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; padding: 5px;">X</td> <td style="border: 1px solid black; padding: 5px;">US, A, 4140299 (Henderson et al) 20 Feb. 1979 See entire document</td> <td style="border: 1px solid black; text-align: center; padding: 5px;">1, 2</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; padding: 5px;">X</td> <td style="border: 1px solid black; padding: 5px;">US, A, 4174907 (Suh et al) 20 Nov. 1979 See entire document</td> <td style="border: 1px solid black; text-align: center; padding: 5px;">1, 2</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; padding: 5px;">X</td> <td style="border: 1px solid black; padding: 5px;">US, A, 3362919 (Rood) 09 Jan. 1968 See entire document</td> <td style="border: 1px solid black; text-align: center; padding: 5px;">1, 2</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; padding: 5px;">X</td> <td style="border: 1px solid black; padding: 5px;">US, A, 3212128 (Carlson et al) 19 Oct. 1965 See entire document</td> <td style="border: 1px solid black; text-align: center; padding: 5px;">1, 2</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; padding: 5px;">Y</td> <td style="border: 1px solid black; padding: 5px;">US, A, 3420506 (Gurley, Jr.) 07 Jan. 1969 See entire document</td> <td style="border: 1px solid black; text-align: center; padding: 5px;">1, 2</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; padding: 5px;">A</td> <td style="border: 1px solid black; padding: 5px;">US, A, 2513562 (Holuba) 04 July 1950</td> <td style="border: 1px solid black;"></td> </tr> <tr> <td style="border: 1px solid black; text-align: center; padding: 5px;">A</td> <td style="border: 1px solid black; padding: 5px;">US, A, 2243309 (Daman et al) 27 May 1941</td> <td style="border: 1px solid black;"></td> </tr> <tr> <td style="border: 1px solid black; text-align: center; padding: 5px;">A</td> <td style="border: 1px solid black; padding: 5px;">US, A, 2843169 (Stein) 15 July 1958</td> <td style="border: 1px solid black;"></td> </tr> </table>			Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	X	US, A, 4482254 (Kessler et al) 13 Nov. 1984 See entire document	1, 2	X	US, A, 4140299 (Henderson et al) 20 Feb. 1979 See entire document	1, 2	X	US, A, 4174907 (Suh et al) 20 Nov. 1979 See entire document	1, 2	X	US, A, 3362919 (Rood) 09 Jan. 1968 See entire document	1, 2	X	US, A, 3212128 (Carlson et al) 19 Oct. 1965 See entire document	1, 2	Y	US, A, 3420506 (Gurley, Jr.) 07 Jan. 1969 See entire document	1, 2	A	US, A, 2513562 (Holuba) 04 July 1950		A	US, A, 2243309 (Daman et al) 27 May 1941		A	US, A, 2843169 (Stein) 15 July 1958	
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IV. CERTIFICATION <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border: 1px solid black; padding: 5px; vertical-align: top;"> Date of the Actual Completion of the International Search <div style="text-align: center; font-size: 1.2em;">05 April 1989</div> </td> <td style="width: 50%; border: 1px solid black; padding: 5px; vertical-align: top;"> Date of Mailing of this International Search Report <div style="text-align: center; font-size: 1.5em;">12 MAY 1989</div> </td> </tr> <tr> <td style="border: 1px solid black; padding: 5px;"> International Searching Authority <div style="text-align: center;">ISA/US</div> </td> <td style="border: 1px solid black; padding: 5px;"> Signature of Authorized Officer <div style="text-align: center;"> Philip R. Coe </div> </td> </tr> </table>			Date of the Actual Completion of the International Search <div style="text-align: center; font-size: 1.2em;">05 April 1989</div>	Date of Mailing of this International Search Report <div style="text-align: center; font-size: 1.5em;">12 MAY 1989</div>	International Searching Authority <div style="text-align: center;">ISA/US</div>	Signature of Authorized Officer <div style="text-align: center;"> Philip R. Coe </div>																										
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	US, A, 3433465 (Szpur) 18 March 1969	
A	US, A, 3972614 (Johansen et al) 03 Aug. 1976	
A	US, A, 4357110 (Hope et al) 02 Nov. 1982	
A	US, A, 4390283 (Meyers) 28 June 1983	
A	US, A, 4403866 (Falcoff et al) 13 Sept. 1983	
A	US, A, 4496244 (Ludwig) 29 Jan. 1985	
A	US, A, 4537512 (Boiron et al) 27 Aug. 1985	
A	US, A, 4720998 (Hogue) 26 Jan. 1988	
A	JP, A, 60-38028 (Isobe) 27 Feb. 1985	
A	JP, A, 62-132527 (Konishiroku Photo) 15 June 1987	

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PUBN-DATE: August 10, 1989

INVENTOR-INFORMATION:

NAME	COUNTRY
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ASSIGNEE-INFORMATION:

NAME	COUNTRY
APPLIED BIOSYSTEMS	US

APPL-NO: US08900346
APPL-DATE: January 27, 1989

PRIORITY-DATA: CH00032188A (January 29, 1988)

INT-CL (IPC): B01F005/04 , B01F007/16 ,
B01F015/02 , B01F015/04 ,
B01F015/06

EUR-CL (EPC): C12M001/02 , B01F007/00

US-CL-CURRENT: 366/142 , 366/149 , 366/165.1 ,
366/181.7

ABSTRACT:

CHG DATE=19940730 STATUS=O>A device for mixing liquids contains a mixing chamber (1) into which a mixing head propelled around a rotationally axis is designed. Between the wall of the mixing chamber and the mixing head (2) a cleft mixing area is created. Through rotation of the mixing head (2) a shear flux of the injected liquids (11, 12, 13, 14) is created which causes mixing. With this active mixing method minute liquid amounts with differing volumes and viscosities can be mixed with high rapidity. The device is especially suitable for the automated analysis of enzyme kinetics.